Original Investigation

Association of Dietary Nitrate Intake With Primary Open-Angle Glaucoma

A Prospective Analysis From the Nurses' Health Study and Health Professionals Follow-up Study

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IMPORTANCE Nitric oxide signaling alterations in outflow facility and retinal blood flow autoregulation are implicated in primary open-angle glaucoma (POAG). Nitric oxide donation has emerged as a POAG therapeutic target. An exogenous source of nitric oxide is dietary nitrates.

OBJECTIVE To evaluate the association between dietary nitrate intake, derived mainly from green leafy vegetables, and POAG.

DESIGN, SETTING, AND PARTICIPANTS We followed up participants biennially in the prospective cohorts of the Nurses' Health Study (63 893 women; 1984-2012) and the Health Professionals Follow-up Study (41 094 men; 1986-2012) at each 2-year risk period. Eligible participants were 40 years or older, were free of POAG, and reported eye examinations.

EXPOSURES The primary exposure was dietary nitrate intake. Information on diet and potential confounders was updated with validated questionnaires.

MAIN OUTCOMES AND MEASURES The main outcome was the incidence of POAG and POAG subtypes; 1483 cases were confirmed with medical records and classified into subtypes defined by intraocular pressure (IOP) (≥22 or <22 mm Hg) or by visual field (VF) loss pattern at diagnosis (peripheral loss only or early paracentral loss). Cohort-specific and pooled multivariable rate ratios (MVRRs) and 95% CIs were estimated.

RESULTS During 1 678 713 person-years of follow-up, 1483 incident cases of POAG were identified. The mean (SD) age for the 1483 cases was 66.8 (8.3). Compared with the lowest quintile of dietary nitrate intake (quintile 1: approximately 80 mg/d), the pooled MVRR for the highest quintile (quintile 5: approximately 240 mg/d) was 0.79 (95% CI, 0.66-0.93; *P* for trend = .02). The dose response was stronger (*P* for heterogeneity = .01) for POAG with early paracentral VF loss (433 cases; quintile 5 vs quintile 1 MVRR = 0.56; 95% CI, 0.40-0.79; *P* for trend < .001) than for POAG with peripheral VF loss only (835 cases; quintile 5 vs quintile 1 MVRR = 0.85; 95% CI, 0.68-1.06; *P* for trend = .50). The association did not differ (*P* for heterogeneity = .75) by POAG subtypes defined by IOP (997 case patients with IOP ≥22 mm Hg: quintile 5 vs quintile 1 MVRR = 0.82; 95% CI, 0.67-1.01; *P* for trend = .11; 486 case patients with IOP <22 mm Hg: quintile 5 vs quintile 1 MVRR = 0.71; 95% CI, 0.53-0.96; *P* for trend = .12). Green leafy vegetables accounted for 56.7% of nitrate intake variation. Compared with consuming 0.31 servings per day, the MVRR for consuming 1.45 or more servings per day was 0.82 for all POAG (95% CI, 0.69-0.97; *P* for trend = .02) and 0.52 for POAG with paracentral VF loss (95% CI, 0.29-0.96; *P* for trend < .001).

CONCLUSIONS AND RELEVANCE Higher dietary nitrate and green leafy vegetable intake was associated with a lower POAG risk, particularly POAG with early paracentral VF loss at diagnosis.

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Elevated intraocular pressure (IOP) and impaired autoregulation of optic nerve blood flow are implicated in primary open-angle glaucoma (POAG).[1-10] Endothelial dysfunction, a key contributor to vascular regulatory impairment, is involved in both processes.[11] The vascular endothelium regulates the microcirculation via vasoactive factors; one potent factor is nitric oxide (NO). In the L-arginine–NO pathway, NO is formed from L-arginine and oxygen by NO synthases (NOSs), such as endothelial NOS (NOS3).[12]

Abundant evidence supports NO's role in POAG pathogenesis.[13] With administration of a systemic NOS inhibitor, differences in ocular blood flow response were observed between cases with POAG and controls.[14] In addition, polymorphisms in *NOS3* (OMIM 163729), the gene for NOS3, were associated with lower blood NO levels[15-19] and POAG.[16,20-22]

As an alternative to the L-arginine–NO pathway, when under hypoxia[23,24] or when NOS may be dysfunctional,[14,16,20,21] which may occur in POAG, exogenous nitrate can be reduced to nitrite[25-27] by commensal bacteria[28-30] and subsequently converted enzymatically or nonenzymatically to NO in tissues[26,31-33] in the nitrate-nitrite-NO pathway.[34-38] Evidence suggests that nitrate or nitrite, precursors for NO, is beneficial for blood circulation.[34,39-42] Dietary nitrate is predominately derived from green leafy vegetables,[43] which contribute approximately 80% of nitrate intake.[29] Although plasma nitrite levels[16,44] or intake of specific vegetables[45,46] have been associated with POAG, to our knowledge, dietary nitrate intake as a specific nutrient has not been evaluated. Therefore, we evaluated dietary nitrate and incident POAG in a longer-than-25-year prospective study of 63 893 women in the Nurses' Health Study (NHS) and 41 094 men in the Health Professionals Follow-up Study (HPFS).

At a Glance

Question: What is the association between dietary nitrate intake, derived mainly from green leafy vegetables, and primary open-angle glaucoma (POAG)?

Findings: Compared with the lowest quintile of dietary nitrate intake (approximately 80 mg/d), the highest quintile (approximately 240 mg/d) was associated with a 21% lower risk of all POAG and 44% lower risk of POAG with early paracentral visual field loss, a subtype of POAG linked to dysfunction in blood flow autoregulation.

Meaning: These findings could have important implications for POAG if the association of higher dietary nitrate and green leafy vegetable intake with a lower POAG risk is confirmed in observational or intervention studies.

Methods

The NHS began in 1976 with 121 700 US registered female nurses (30-55 years old) who completed a mailed questionnaire.[47] The HPFS began in 1986 with 51 529 US male health professionals (dentists, veterinarians, pharmacists, optometrists, osteopaths, and podiatrists) aged 40 to 75 years.[48] Participants have been followed up biennially with mailed questionnaires of health, diet, and diseases, such as glaucoma. Follow-up rates were high (>85%). The human research committees of Brigham & Women's Hospital, Massachusetts Eye and Ear Infirmary, and the Harvard School of Public Health approved this study. Participants' return of a completed questionnaire was accepted as implied informed consent by the human research committees.

The first detailed assessment of diet with a semiquantitative food frequency questionnaire (SFFQ) was conducted in 1984 for the NHS and 1986 for the HPFS; thus, these dates are the baseline years. Participants contributed person-years in approximately 2-year units from the return date of 1 questionnaire to that of another until the earliest occurrence of a glaucoma report, cancer, death, loss to follow-up, or the end of study in 2012. Eligible participants were 40 years or older (when glaucoma risk increases) and reported undergoing an eye examination in the 2-year risk period (to minimize possible detection bias).

We excluded at baseline the following NHS and HPFS participants, respectively: 47 512 and 1596 who did not respond to baseline SFFQs or had outlying total caloric intakes; 4011 and 1927 with prevalent cancers, excluding nonmelanoma skin cancer, because cancer diagnoses could alter diet; 902 and 1035 with prevalent glaucoma; 450 and 1874 lost to follow-up from 1976 to 1984 (NHS) or less than 2 years of baseline (NHS and HPFS); and 2391 and 3251 who never reported an eye examination during follow-up. After these exclusions, 66 435 and 41 846 NHS and HPFS participants, respectively, were eligible; however, at the beginning of each 2-year risk period, we applied additional provisional exclusions for age and eye examination status. For example, for the 1984-1986 (NHS) and 1986-1988 (HPFS) risk periods, respectively, only 45 955 and 29 039 contributed person-years after we provisionally excluded participants (20 480 and 12 807) who were younger than 40 years and reported no eye examinations. In later periods, those provisionally excluded were allowed in analyses if they met eligibility criteria during follow-up. Thus, during the study period, 63 893 and 41 094 NHS and HPFS participants, respectively, ever contributed person-years.

Ascertainment of Cases With POAG and Classification of POAG by IOP and VF Loss Pattern

We included 1483 cases with confirmed incident POAG (1000 women and 483 men). Glaucoma case ascertainment occurred biennially when we asked about eye examinations and physician diagnoses of glaucoma. For those self-reporting glaucoma, we sought permission to contact eye care professionals, who were requested to send all visual fields (VFs) with medical records or a completed glaucoma questionnaire with items on maximal IOP, status of the filtration apparatus, optic nerve structural information, ophthalmic surgery, and VF loss. Records were reviewed by a glaucoma specialist (L.R.P.), masked to participants' diet, to confirm POAG cases using standardized criteria.

Cases had to be appraised as definite or probable POAG. For definite POAG cases (>70% of all cases), the following criteria were required: (1) gonioscopy in which the filtration angle was not occludable in either eye; (2) slitlamp biomicroscopy with no signs in either eye of pigment dispersion syndrome, uveitis, exfoliation syndrome, trauma, or rubeosis; and (3) reproducible VF defects consistent with POAG on 2 or more

reliable tests. For probable POAG cases, the slitlamp examination and VF criteria were also required, but documentation of pupil dilation without subsequent adverse events was accepted in lieu of gonioscopy. For VF defects, the type of perimetry was not restricted; however, full static threshold testing was documented in 95% and kinetic VFs in less than 1%. For static threshold or suprathreshold tests, the following reliability definitions were used: fixation loss of 33% or less, false-positive rate of 20% or less, and false-negative rate of 20% or less. For kinetic VFs, a VF test result was considered reliable unless the examiner noted to the contrary.

New glaucoma diagnoses were self-reported by 8611 and 3791 NHS and HPFS participants, respectively, and these diagnoses were confirmed as various types of glaucoma or suspicion of glaucoma in 5487 (63.7%) and 2058 (54.3%): potential POAG with VF loss (2290 [26.6%] and 985 [26.0%]), elevated IOP or optic disc cupping only (1559 [18.1%] and 612 [16.1%]), and other types of glaucoma or suspicion of glaucoma (1639 [19.0%] and 461 [12.2%]). The remaining diagnoses (3124 [36.3%] and 1733 [45.7%]) were unconfirmed because participants (689 [8.0%] and 563 [14.9%]) or eye care professionals (435 [5.1%] and 204 [5.4%]) were unreachable, participants denied permission for record review (984 [11.4%] and 362 [9.7%]), participants indicated the report was erroneous (835 [9.7%] and 547 [14.4%]), or eye care professionals refuted the glaucoma diagnosis (181 [2.1%] and 57 [1.5%]). Among those diagnoses classified as potential POAG with VF loss, we included only the definite or probable POAG cases (NHS, 1000 and HPFS, 483); other confirmed and unconfirmed self-reports were censored as of the diagnosis date.

For secondary analyses, we classified cases into subtypes by IOP and by VF loss pattern at diagnosis. We defined subtypes of high-tension (n = 997; 651 and 346 of NHS and HPFS participants, respectively) and normal-tension POAG (n = 486; 349 and 137 of NHS and HPFS participants, respectively) as those with a maximum untreated IOP of 22 mm Hg or higher or less than 22 mm Hg, respectively. We defined subtypes by VF loss pattern: those with peripheral VF loss only (n = 835; 576 and 259 of NHS and HPFS participants, respectively) or early paracentral VF loss (n = 433; 288 and 145 of NHS and HPFS participants, respectively) or undetermined VF loss (n = 215; 136 and 79 of NHS and HPFS participants, respectively) with a previously described method.[49] For a case with peripheral VF loss only, nasal step, temporal wedge, or Bjerrum scotoma was present with no paracentral loss. For a case with early paracentral loss, there was paracentral loss only or paracentral loss with VF loss in the Bjerrum area and/or nasal step area in the same hemifield but without any temporal wedge loss. We included the latter paracentral group because those with only paracentral loss were uncommon (approximately 21%), whereas those with clear paracentral loss frequently also had peripheral loss. Cases (n = 215) with undetermined VF loss (ie, VF loss in the paracentral and any temporal wedge regions in the same eye or paracentral loss in 1 hemifield with peripheral loss only in the other hemifield) were censored. The proportion of those with normal-tension POAG was 38.3% in those with early paracentral VF loss and 28.8% in those with peripheral VF loss only.

Measurement of Intake of Nitrate and Vegetable Sources of Nitrate

Validated SFFQs were administered every 2 to 4 years. The 1984 NHS SFFQ included 116 items, and similar versions were used from 1986 in the NHS (126 items) and HPFS (131 items). The SFFQ inquires about the average intake of a serving of a food or beverage in the preceding year, with intake choices from never or less than 1 per month to 6 or more per day. To convert responses into average daily intakes of nitrate, nutrient content information of each food obtained from updated US Department of Agriculture food composition[50] was used and combined with frequency information.

For primary analyses, we examined daily intake of nitrate and vegetables. Vegetables included celery and others in 4 groups: green leafy vegetables (iceberg lettuce; romaine lettuce; kale, mustard, or chard; cooked spinach; and raw spinach), cruciferous vegetables (kale, mustard, or chard; broccoli; cabbage or coleslaw; cauliflower; and Brussels sprouts), root vegetables (beets, potatoes, onions, carrots, yams or sweet potatoes), and tomato-based foods (tomatoes, tomato sauce, tomato juice).

We evaluated updated cumulatively averaged intakes, which better represent long-term exposure and have less random measurement error.[51] With cumulative averaging, the average of all available information was used (eg, in the NHS in 1984, the 1984 nitrate value was used; in 1986, the average of the 1984 and 1986 values was used). Intakes of other dietary factors (eg, other antioxidants, caffeine, alcohol, folate, or flavonoids) were similarly derived.

Validity of SFFQ Assessment of Nitrate and Vegetable Sources

The reproducibility and validity of the SFFQ have been reported previously.[52,53] In a biomarker study[54] among 630 participants, being in the highest tertile of dietary nitrate intake based on the SFFQ was associated with a 3.18-mmol/L increase in plasma nitrate (P = .1). In 127 participants who completed both the SFFQ and multiple weighed dietary records,[55] the SFFQ performed reasonably well, with a mean correlation with dietary record values of 0.46 across vegetables: from 0.25 for kale, mustard, or chard greens to 0.73 for lettuce.

Statistical Analysis

For analyses of nitrate intake, intake values were total energy adjusted using the residual method.[56] For food analyses, we adjusted for cumulatively updated total calories.

We calculated incidence rates of POAG by dividing the incident cases by person-years accrued for each intake category (quintiles). For multivariable analyses, we conducted Cox proportional hazards regression analysis stratified by age in months and the specific 2-year period at risk[57] while controlling for potential glaucoma risk factors. We derived incidence rate ratios and 95% CIs. We conducted tests for trend by evaluating the significance of a variable representing quintile median values.

Potential covariates were updated biennially from baseline: glaucoma family history, African heritage, body mass index (calculated as weight in kilograms divided by height in meters squared), pack-years of smoking, hypertension, diabetes mellitus, physical activity (metabolic equivalent hours per

Table 1. Person-time Characteristics by Total Nitrate Intake During the Follow-up Period in the Nurses' Health Study (1984-2012) and the Health Professionals Follow-up Study (1986-2012)

Characteristic	Quintile 1		Quintile 3		Quintile 5	
	Women (n = 113 917)	Men (n = 56 662)	Women (n = 113 424)	Men (n = 56 348)	Women (n = 113 584)	Men (n = 56 272)
Age, mean (SD), y	61.0 (10.2)	61.5 (10.6)	62.3 (10.0)	62.7 (10.5)	63.4 (9.7)	63.7 (10.2)
Dietary intake, mean (SD)						
Total nitrate intake, mg/d	77.3 (17.1)	78.6 (18.1)	141.9 (9.0)	148.2 (10.7)	261.0 (75.3)	279.5 (89.8)
Green leafy vegetables, servings per d	0.3 (0.2)	0.3 (0.2)	0.8 (0.2)	0.7 (0.2)	1.5 (0.6)	1.4 (0.6)
Cruciferous vegetables, servings per d	0.3 (0.2)	0.3 (0.2)	0.5 (0.2)	0.5 (0.3)	0.7 (0.4)	0.8 (0.5)
Root vegetables, servings per d	0.9 (0.4)	0.9 (0.5)	1.1 (0.5)	1.1 (0.5)	1.3 (0.6)	1.3 (0.7)
Tomato-based foods, servings per d	0.4 (0.2)	0.4 (0.3)	0.6 (0.3)	0.6 (0.3)	0.8 (0.4)	0.8 (0.5)
Celery, servings per d	0.1 (0.1)	0.1 (0.1)	0.2 (0.2)	0.2 (0.2)	0.4 (0.4)	0.3 (0.3)
Total caloric intake, kcal/d	1723.4 (450.3)	1967.2 (564.0)	1781.0 (438.5)	2011.9 (540.0)	1716.0 (444.1)	1946.4 (542.8)
Alcohol intake, g/d	5.2 (9.5)	10.8 (14.8)	6.1 (9.1)	11.4 (13.4)	6.2 (8.8)	10.3 (12.0)
Caffeine intake, mg/d	275.0 (200.0)	249.3 (225.2)	264.8 (185.4)	224.9 (207.7)	263.3 (193.0)	214.9 (209.3)
Total carotenoid intake, IU/d	6045.8 (2951.2)	6744.1 (3952.3)	9192.8 (3678.9)	10 289.4 (5101.9)	13 974.2 (6338.7)	16 411.0 (9393.3)
Total folate intake, µg/d	393.7 (184.5)	472.9 (231.1)	447.9 (182.0)	536.1 (231.3)	530.3 (201.6)	639.3 (266.6)
Total flavonoid intake, mg/d	318.2 (283.7)	293.8 (246.5)	342.3 (251.0)	342.3 (237.6)	383.0 (272.0)	391.6 (255.4)
Vitamin A intake, IU/d	9931.5 (4712.9)	11 460.8 (6350.9)	13 368.4 (5380.1)	15 142.8 (7555.8)	18 618.7 (8092.1)	21 672.4 (11 477.7)
Vitamin C intake, mg/d	276.5 (279.0)	350.6 (371.8)	336.0 (301.1)	429.4 (409.0)	432.7 (368.0)	545.1 (474.9)
Vitamin E intake, mg/d	46.2 (67.3)	55.8 (79.6)	53.3 (70.8)	64.3 (83.6)	65.5 (83.2)	78.4 (97.4)
Age-adjusted characteristics, %						
Family history of glaucoma	13.1	11.0	13.5	11.5	13.8	11.6
African ancestry	0.7	0.5	0.8	0.6	1.8	1.1
Self-reported diagnosis						
Diabetes mellitus	7.3	6.3	7.0	6.2	7.1	7.8
Hypertension	42.1	36.6	41.8	36.2	42.1	37.1
≥30 Pack-years of smoking	20.1	19.1	16.0	16.0	16.1	15.3
Body mass index ≥30	19.6	11.4	18.8	10.6	18.2	11.3
Physical activity (top 25th percentile)	16.8	20.2	25.3	25.7	34.5	31.7

week), number of eye examinations reported during follow-up, multivitamin use, and, among women, age at menopause and postmenopausal hormone use. In addition, main multivariable models (model 1) included other dietary components: cumulatively updated intake categories of total calories, alcohol, and caffeine. In additional multivariable models (model 2), we further adjusted for intake of folate, vitamin A, and antioxidants (alpha carotene, beta carotene, beta cryptoxanthin, lycopene, lutein or zeaxanthin, other carotenoids, flavonoids, and vitamins C and E).

We analyzed cohort-specific data separately and performed tests for heterogeneity to check for appropriateness of pooling the results. We then pooled the results using meta-analytic methods that incorporated random effects.[58]

Secondary Analyses

We performed several secondary analyses. First, we evaluated nitrate intake only as of baseline or as of the most recent questionnaire. Second, we separately analyzed the risks of high- vs normal-tension POAG and POAG with peripheral VF loss only vs early paracentral loss. When testing whether the associations between nitrate and 1 POAG subtype are different from those with another subtype, we combined the data sets into 1, then conducted Cox proportional hazards regression analyses that further stratified on the 2 cohorts (to allow for differing hazard functions) and used the Lunn-McNeil approach[59] to derive the *P* for heterogeneity.

Results

During 1 678 713 person-years of follow-up, we identified 1483 incident cases. The mean (SD) age for the 1483 cases was 66.8 (8.3). Highest consumers of dietary nitrate consumed more antioxidants (carotenoids, vitamin C, vitamin E), flavonoids, folate, and vitamin A; exercised more; and were more frequently African American (**Table 1**). They were also leaner and smoked less. These differences were adjusted for in multivariable analyses.

In nitrate analyses, cohort-specific results were not heterogeneous and thus were pooled. Age-adjusted and multivariable analyses revealed similar associations (**Table 2**). Compared with the lowest quintile (quintile 1)

Table 2. Primary Open-Angle Glaucoma by Quintiles of Nitrate Intake in the Nurses' Health Study (1984-2012) and the Health Professionals Follow-up Study (1986-2012)[a]

Variable	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* Value for Trend
Cumulatively Updated Diet						
Women						
Median nitrate intake, mg/d	80	114	142	175	238	...
No. of cases	210	173	207	199	211	...
Person-years	227 054	227 827	226 545	227 053	226 982	...
RR (95% CI)						
Age adjusted	1 [Reference]	0.79 (0.64-0.96)	0.89 (0.73-1.08)	0.82 (0.67-0.99)	0.85(0.70-1.04)	.28
Model 1[b]	1 [Reference]	0.77 (0.63-0.95)	0.85 (0.70-1.03)	0.78 (0.64-0.95)	0.81 (0.67-0.99)	.12
Model 2	1 [Reference]	0.74 (0.59-0.92)	0.77 (0.61-0.97)	0.67 (0.51-0.86)	0.67 (0.50-0.90)	.02
Men						
Median nitrate intake, mg/d	81	117	148	185	254	...
No. of cases	98	89	101	111	84	...
Person-years	108 530	109 243	108 596	108 704	108 180	...
RR (95% CI)						
Age adjusted	1 [Reference]	0.92 (0.69-1.24)	1.02 (0.76-1.35)	1.11 (0.84-1.47)	0.78 (0.57-1.05)	.21
Model 1[b]	1 [Reference]	0.90 (0.67-1.22)	0.97 (0.72-1.30)	1.08 (0.81-1.43)	0.72 (0.53-0.98)	.09
Model 2[c]	1 [Reference]	0.88 (0.64-1.21)	0.94 (0.67-1.32)	1.03 (0.71-1.49)	0.66 (0.42-1.03)	.11
Pooled[d]						
Model 1[b]	1 [Reference]	0.81 (0.69-0.96)	0.88 (0.75-1.04)	0.90 (0.66-1.23)	0.79 (0.66-0.93)	.02
Model 2[c]	1 [Reference]	0.78 (0.65-0.94)	0.82 (0.67-0.99)	0.81 (0.53-1.24)	0.67 (0.52-0.85)	.01
Baseline[e]						
Pooled[d]						
Model 1[b]	1 [Reference]	0.96 (0.81-1.13)	0.94 (0.77-1.15)	1.04 (0.73-1.47)	0.88 (0.74-1.04)	.21
Model 2[c]	1 [Reference]	0.98 (0.82-1.18)	1.00 (0.69-1.44)	1.08 (0.59-1.97)	0.90 (0.59-1.36)	.64
Most Recent[e]						
Pooled[d]						
Model 1[b]	1 [Reference]	1.09 (0.92-1.30)	1.03 (0.77-1.37)	1.01 (0.85-1.20)	0.91 (0.76-1.09)	.13
Model 2[c]	1 [Reference]	1.05 (0.87-1.26)	1.01 (0.66-1.57)	1.00 (0.71-1.40)	0.83 (0.65-1.05)	.08

Abbreviations: ellipses, data not applicable; RR, relative risk.

[a] Intake calculated using cumulative average (ie, average of all available intake data from food frequency questionnaires completed before each 2-year period at risk).

[b] All multivariable analyses were stratified by age in months and period at risk, and they were adjusted for the following variables: ancestry (African American, non-African heritage); family history of glaucoma; self-reported history of hypertension; diabetes; body mass index (22-23, 24-25, 26-27, 28-29, and ≥30); cumulatively averaged intakes of total energy (kilocalories per day in quintiles); alcohol (grams per day in categories of 0-4, 5-14, 15-29, and ≥30 g/d); and caffeine (milligrams per day in quintiles); pack-years of smoking (1-9, 10-19, 20-29, and ≥30 pack-years); physical activity (quartiles of metabolic equivalent hours per week); number of eye examinations reported during follow-up; multivitamin use (nonuser, past user, or current user), and in the Nurses' Health Study only, they were additionally adjusted for age at menopause (20-44, 45-50, 50-54, ≥54 years) and postmenopausal hormone status (premenopausal, current user, past user, or nonuser).

[c] Includes all variables in model 1 with additional adjustment for other nutrients: quintiles of alpha carotene, beta carotene, beta cryptoxanthin, lycopene, lutein or zeaxanthin, other carotenoids, folate, flavonoid, and vitamins A, C, and E.

[d] Pooled results were calculated using DerSimonian and Laird methods with random effects; *P* for heterogeneity >.05 between cohorts for all *P* values for linear trend results.

[e] Baseline diet refers to diet as of 1984 in women and 1986 in men; most recent diet refers to the intake as of the food frequency questionnaire immediately before each 2-year period at risk.

of approximately 80 mg/d of nitrate, the pooled multivariable relative risk (MVRR) of POAG in the main model (model 1) was 0.81 (95% CI, 0.69-0.96) for quintile 2, 0.88 (95% CI, 0.75-1.04) for quintile 3, 0.90 (95% CI, 0.66-1.23) for quintile 4, and 0.79 (95% CI, 0.66-0.93) for quintile 5 (*P* for trend = .02) (Table 2). When other dietary factors (model 2) were also adjusted for, similar inverse associations were observed (pooled MVRR for quintile 5 vs quintile 1, 0.67; 95% CI, 0.52-0.85; *P* for trend = .01).

When we explored the timing of intake, no association was found between nitrate intake only at baseline and intake at the most recent SFFQ (pooled MVRR for model 1, 0.88; 95% CI, 0.74-1.04; *P* for trend = .21 for baseline intake and 0.91; 95% CI, 0.76-1.09; *P* for trend = .13 for most recent intake for quintile 5 compared with quintile 1).

When nitrate intake with POAG subtypes characterized by IOP at diagnosis was evaluated (**Table 3**), we observed similar associations, and the *P* for heterogeneity was .75. However, we observed differences (*P* for heterogeneity = .01) in associations by VF subtypes (pooled MVRR for POAG with peripheral VF loss only, 0.85; 95% CI, 0.68-1.06; *P* for trend = .50; pooled MVRR for POAG with early paracentral VF loss, 0.56; 95% CI, 0.40-0.79; *P* for trend < .001 for quintile 5 compared with quintile 1).

Table 3. Subtypes of Primary Open-Angle Glaucoma by Quintiles of Nitrate Intake in the Nurses' Health Study (1984-2012) and the Health Professionals Follow-up Study (1986-2012)[a]

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* Value for Trend	*P* Value for Heterogeneity[b]
Subtypes Defined by IOP[c]							
High-tension glaucoma (IOP ≥22 mm Hg) (n = 998)							.75
Women							
No. of cases	133	117	138	121	142	...	
Multivariable RR (95% CI)[d]	1 [Reference]	0.83 (0.64-1.06)	0.89 (0.70-1.14)	0.75 (0.58-0.96)	0.87 (0.68-1.11)	.29	
Men							
No. of cases	68	62	75	82	59	...	
Multivariable RR (95% CI)[d]	1 [Reference]	0.89 (0.62-1.27)	1.01 (0.72-1.43)	1.11 (0.79-1.56)	0.73 (0.51-1.06)	.20	
Pooled[e]							
Multivariable RR (95% CI)	1 [Reference]	0.85 (0.69-1.04)	0.93 (0.76-1.13)	0.90 (0.61-1.32)	0.82 (0.67-1.01)	.11	
Normal-tension glaucoma (IOP <22 mm Hg) (n = 487)							
Women							
No. of cases	77	56	69	78	69	...	
Multivariable RR (95% CI)[d]	1 [Reference]	0.68 (0.48-0.96)	0.77 (0.55-1.07)	0.82 (0.59-1.13)	0.71 (0.51-1.00)	.22	
Men							
No. of cases	30	27	26	29	25	...	
Multivariable RR (95% CI)[d]	1 [Reference]	0.90 (0.52-1.56)	0.84 (0.48-1.47)	0.98 (0.57-1.70)	0.72 (0.41-1.28)	.34	
Pooled[e]							
Multivariable RR (95% CI)	1 [Reference]	0.73 (0.55-0.98)	0.79 (0.59-1.05)	0.86 (0.65-1.13)	0.71 (0.53-0.96)	.12	
Subtypes Defined by Initial VF Loss Pattern[f]							
POAG with peripheral VF loss only (n = 836 cases)							.01
Women							
No. of cases	120	91	121	120	124	...	
Multivariable RR (95% CI)[d]	1 [Reference]	0.71 (0.54-0.93)	0.86 (0.67-1.12)	0.83 (0.64-1.07)	0.84 (0.65-1.09)	.60	
Men							
No. of cases	47	47	58	62	45	...	
Multivariable RR (95% CI)[d]	1 [Reference]	1.01 (0.67-1.55)	1.20 (0.80-1.80)	1.29 (0.86-1.92)	0.87 (0.57-1.34)	.67	
Pooled[e]							
Multivariable RR (95% CI)	1 [Reference]	0.82 (0.58-1.15)	0.98 (0.72-1.34)	1.00 (0.65-1.54)	0.85 (0.68-1.06)	.50	
POAG with early paracentral VF loss (n = 433 cases)							
Women							
No. of cases	61	64	58	56	49	...	
Multivariable RR (95% CI)[d]	1 [Reference]	0.95 (0.66-1.35)	0.79 (0.55-1.14)	0.74 (0.51-1.07)	0.64 (0.43-0.94)	.01	
Men							
No. of cases	35	28	28	32	22		
Multivariable RR (95% CI)[d]	1 [Reference]	0.79 (0.47-1.34)	0.72 (0.42-1.22)	0.84 (0.50-1.39)	0.44 (0.25-0.78)	.01	
Pooled[e]							
Multivariable RR (95% CI)	1 [Reference]	0.89 (0.67-1.20)	0.77 (0.57-1.04)	0.77 (0.57-1.04)	0.56 (0.40-0.79)	<.001	

Abbreviations: ellipses, data not applicable; IOP, intraocular pressure; POAG, primary open-angle glaucoma; RR, relative risk; VF, visual field.

[a] Intake calculated using cumulative average (ie, average of all available intake data from food frequency questionnaires completed before each 2-year period at risk).

[b] To test whether the associations between nitrate and 1 POAG subtype is significantly different from those with another subtype, we combined the 2 data sets into 1, then conducted Cox regression analyses that stratified on the 2 cohorts, which allowed for the baseline hazard function to be different in the cohorts; we then used the Lunn-McNeil approach[59] to test for heterogeneity in associations and derived the *P* for heterogeneity.

[c] Based on the maximum untreated IOP at diagnosis.

[d] All multivariable analyses were adjusted for the same variables as those in model 1 in Table 2.

[e] Pooled results were calculated using Dersimonian and Laird methods with random effects.

[f] Based on VF loss pattern as of the earliest reliable VF at diagnosis that was reproduced at the latest reliable VF. Cases (n = 216) with advanced VF loss at diagnosis who could not be categorized based on initial presenting VF loss as either peripheral VF loss only or early paracentral VF loss were censored during analyses. See the Methods section to determine how cases were categorized according to initial presenting VF loss.

Table 4. Quintiles of Daily Servings of Foods High in Nitrate in Relation to All POAG and Para-POAG in the Nurses' Health Study (1984-2012) and the Health Professionals Follow-up Study (1986-2012)[a]

Food	Variability in Nitrate Explained, %	Outcome	Pooled Multivariable RR (95% CI)					*P* Value for Trend
			Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Green leafy vegetables[b]	56.7	Median	0.31	0.56	0.75	1.00	1.45	...
		All POAG	1 [Reference]	0.95 (0.81-1.12)	0.90 (0.66-1.22)	0.93 (0.61-1.44)	0.82 (0.69-0.97)	.02
		Para-POAG	1 [Reference]	0.89 (0.57-1.41)	0.67 (0.49-0.92)	0.69 (0.51-0.93)	0.52 (0.29-0.96)	<.001
Iceberg lettuce	23.2	Median	0.11	0.25	0.43	0.55	0.86	...
		All POAG	1 [Reference]	1.00 (0.83-1.20)	1.03 (0.87-1.23)	0.88 (0.69-1.13)	0.89 (0.75-1.06)	.06
		Para-POAG	1 [Reference]	1.03 (0.63-1.70)	0.89 (0.56-1.42)	0.72 (0.41-1.26)	0.69 (0.49-0.97)	.001
Romaine lettuce	17.4	Median	0	0.05	0.12	0.25	0.55	...
		All POAG	1 [Reference]	0.93 (0.77-1.11)	0.98 (0.82-1.15)	0.89 (0.76-1.05)	0.87 (0.74-1.03)	.11
		Para-POAG	1 [Reference]	1.10 (0.61-1.97)	1.17 (0.48-2.89)	1.01 (0.66-1.55)	0.71 (0.29-1.75)	.19
Kale, mustard, or chard greens[b]	6.0	Median	0	0.01	0.04	0.07	0.13	...
		All POAG	1 [Reference]	1.02 (0.84-1.24)	0.96 (0.77-1.19)	0.92 (0.61-1.38)	0.72 (0.54-0.95)	.08
		Para-POAG	1 [Reference]	0.97 (0.68-1.39)	1.03 (0.70-1.53)	1.09 (0.66-1.80)	0.33 (0.16-0.69)	.01
Cruciferous vegetables[b]	15.9	Median	0.16	0.29	0.42	0.58	0.90	...
		All POAG	1 [Reference]	1.28 (1.08-1.52)	1.10 (0.87-1.38)	1.10 (0.92-1.32)	1.12 (0.94-1.35)	.93
		Para-POAG	1 [Reference]	1.23 (0.90-1.69)	1.04 (0.75-1.45)	1.15 (0.83-1.59)	1.02 (0.73-1.43)	.72
Root vegetables[b,c]	9.7	Median	0.50	0.77	1.00	1.29	1.76	...
		All POAG	1 [Reference]	0.88 (0.73-1.05)	0.83 (0.63-1.09)	0.94 (0.64-1.40)	0.87 (0.56-1.36)	.77
		Women	1 [Reference]	0.88 (0.71-1.09)	0.93 (0.75-1.15)	1.13 (0.91-1.40)	1.07 (0.85-1.35)	.12
		Men	1 [Reference]	0.88 (0.64-1.21)	0.70 (0.50-0.97)	0.76 (0.54-1.05)	0.68 (0.48-0.96)	.04
		Para-POAG	1 [Reference]	0.74 (0.48-1.15)	0.83 (0.59-1.15)	0.85 (0.27-2.65)	0.84 (0.34-2.08)	.89
		Women	1 [Reference]	0.88 (0.58-1.35)	0.84 (0.55-1.28)	1.48 (0.99-2.20)	1.29 (0.84-1.98)	.03
		Men	1 [Reference]	0.56 (0.31-1.02)	0.80 (0.46-1.37)	0.46 (0.25-0.86)	0.51 (0.27-0.96)	.04
Celery	6.5	Median	0.02	0.07	0.11	0.23	0.44	...
		All POAG	1 [Reference]	0.88 (0.73-1.06)	0.88 (0.63-1.22)	0.92 (0.77-1.10)	0.93 (0.78-1.11)	.74
		Para-POAG	1 [Reference]	0.80 (0.56-1.14)	0.76 (0.55-1.05)	0.72 (0.47-1.13)	0.93 (0.68-1.27)	.79
Tomato-based foods[b]	4.0	Median	0.21	0.37	0.53	0.71	1.02	
		All POAG	1 [Reference]	0.97 (0.83-1.14)	0.89 (0.75-1.05)	0.86 (0.73-1.02)	0.90 (0.75-1.07)	.14
		Para-POAG	1 [Reference]	0.94 (0.69-1.28)	0.89 (0.65-1.22)	1.02 (0.57-1.80)	0.84 (0.60-1.17)	.37

Abbreviations: ellipses, data not applicable; Para-POAG, primary open-angle glaucoma with early paracentral visual field loss; POAG, primary open-angle glaucoma; RR, relative risk.

[a] Intake calculated using cumulative average (ie, average of all available intake data from food frequency questionnaires completed before each 2-year period at risk). Pooled results were calculated using Dersimonian and Laird methods with random effects; *P* for heterogeneity >.05 between cohorts for all *P* values for linear trend results. All multivariable analyses were adjusted for the same variables as those in model 1 in Table 2.

[b] Kale, mustard, or chard greens were included in both the green leafy vegetable and cruciferous vegetable categories. Green leafy vegetables included iceberg lettuce; romaine lettuce; kale, mustard, or chard greens; cooked spinach; and raw spinach. Cruciferous vegetables included broccoli; cabbage or coleslaw; cauliflower; kale, mustard, or chard greens; and Brussels sprouts. Root vegetables included potatoes, beets, onions, carrots, and yams or sweet potatoes. Tomato-based foods included whole tomatoes, tomato sauce, and tomato juice.

[c] The results for women and men were heterogeneous (*P* for heterogeneity was .01 for all POAG and.004 for all para-POAG); thus, cohort-specific results are also provided. For all other food groups, the *P* for heterogeneity was >.50.

For specific foods and food groups (**Table 4**), when compared with those consuming a median of 0.31 servings per day of green leafy vegetables (quintile 1), the pooled MVRR for 1.45 servings per day (quintile 5) was 0.82 (95% CI, 0.69-0.97; *P* for trend = .02) for overall POAG and 0.52 (95% CI, 0.29-0.96; *P* for trend < .001) for POAG with early paracentral VF loss. Among green leafy

vegetables, the pooled MVRR for quintile 5 vs quintile 1 ranged from 0.72 to 0.89 for overall POAG; for POAG with early paracentral VF loss, the pooled MVRRs were 0.69 (95% CI, 0.49-0.97; *P* for trend = .001) for iceberg lettuce, 0.71 (95% CI, 0.29-1.75; *P* for trend = .19) for romaine lettuce, and 0.33 (95% CI, 0.16-0.69; *P* for trend = .01) for kale, mustard, or chard greens. Associations were not observed with other nitrate-contributing food or food groups except root vegetables. Inverse associations were observed for root vegetables in men only (*P* for heterogeneity = .01): in men, the pooled MVRR for consuming 1.76 servings per day (quintile 5) compared with 0.50 servings per day (quintile 1) was 0.68 (95% CI, 0.48-0.96; *P* for trend = .04) for overall POAG and 0.51 (95% CI, 0.27-0.96; *P* for trend = .04) for POAG with early paracentral VF loss.

Discussion

Greater intake of dietary nitrate and green leafy vegetables was associated with a 20% to 30% lower POAG risk; the association was particularly strong (40%-50% lower risk) for POAG with early paracentral VF loss at diagnosis, for which ocular vascular dysregulation has been implicated.[60]

Evidence suggests a key role of the NO system in POAG pathogenesis; alterations of this system may dysregulate ocular blood flow[14,61] and IOP.[62-68] Elevated IOP was observed in a murine POAG model after the gene for soluble guanylate cyclase, the NO intracellular receptor, was knocked out.[69] Nitric oxide may regulate IOP by mediating aqueous humor outflow. In an in vitro study,[70] glaucomatous Schlemm canal cells produced negligible NO after shear stress compared with nonglaucomatous cells. Thus, exogenous NO donators are emerging as new glaucoma therapeutics.[13]

The nitrate-nitrite-NO pathway may be an important alternative source of NO in POAG. One lettuce serving can yield more NO than that generated daily via the L-arginine–NO pathway.[71] Tissue NO bioavailability and cerebral blood flow can increase with nitrate salts[72,73] and nitrate-rich beet juice supplementation.[74-79] Therefore, dietary nitrate supplementation represents a practical method to increase NO levels. Indeed, across the 2 cross-sectional studies in all (95 cases among 1155 total)[45] or only African American (77 cases among 587 total)[46] women in the Study of Osteoporotic Fractures, the only vegetable that was consistently inversely associated with POAG was kale or collard greens: 1 serving or more per month of kale or collard greens was significantly associated with 55% to 70% reduced odds of POAG.

The stronger inverse association of POAG with early paracentral VF loss is consistent with evidence that this subtype is more strongly associated with vascular dysregulation.[69,80,81] The blood vessels for the inferior paracentral fibers are in the macula vulnerability zone[82] and make more acute arcuate turns than others, creating greater shear forces that could compromise local blood flow.[61] In addition, among patients with glaucoma and autonomic dysfunction or abnormal peripheral microcirculation, paracentral VF defects were more common[80]; one hypothesis is that central fibers may have relatively high oxygen demand and thus be more vulnerable to vascular dysregulation.[83,84] Furthermore, genetic loci related to the NO pathway (eg, *CAV1/CAV2*[85] [OMIM 601047/601048] and *GUCY1A3/GUCY1B3* [OMIM 139396/139397] regions[69]) are most strongly associated with POAG with paracentral loss. Thus, further studies of exogenous nitrate and POAG with paracentral VF loss are warranted.

This was a large prospective study with 1483 incident cases identified from 63 893 women and 41 094 men followed up for more than 25 years, with high follow-up rates. With repeated questionnaires, we evaluated nitrate intake and POAG in various ways (ie, baseline, recent, and cumulative intake) and controlled for numerous updated POAG risk factors.

Our study had a few limitations. We could not conduct repeated eye examinations; consequently, we relied on questionnaires and medical records for disease confirmation. Our case ascertainment method had low sensitivity; however, methodologically, incidence rate ratios can still be valid if the case definition is highly specific and the ascertainment method is unrelated to exposure.[86] Our case definition was highly specific with the requirement of reproducible VF loss, the case ascertainment was unlikely to be related to diet, and we required eye examinations at each follow-up cycle to minimize bias. Another limitation was residual confounding by other dietary factors because nitrate-rich vegetables may have other nutrients. However, we adjusted for intake of other nutrients, and the inverse associations were robust. We may have had some misclassification of nitrate intake from errors in participants recall and because vegetable nitrate content can vary by soil conditions, season, and storage[87,88]; however, these factors would have biased associations toward the null. In addition, because both cohorts are more than 90% white, our results may not be generalizable; however, in a study of African Americans, kale and collard intake was also associated with a lower POAG risk.[46] Finally, these data represent findings from the first population-based observational study; thus, the association between dietary nitrate consumption and POAG should be interpreted cautiously and confirmed.

Conclusions

Greater intake of dietary nitrate, an exogenous NO source, was associated with a lower risk of POAG, particularly POAG with early paracentral VF loss. These results, if confirmed in observational and intervention studies, could have important public health implications.

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REFERENCES

1. Moore D, Harris A, Wudunn D, Kheradiya N, Siesky B. Dysfunctional regulation of ocular blood flow: a risk factor for glaucoma? *Clin Ophthalmol*. 2008;2(4):849-861.

2. Ulrich A, Ulrich C, Barth T, Ulrich WD. Detection of disturbed autoregulation of the peripapillary choroid in primary open angle glaucoma. *Ophthalmic Surg Lasers*. 1996;27(9):746-757.

3. Gugleta K, Orgül S, Hasler PW, Picornell T, Gherghel D, Flammer J. Choroidal vascular reaction to hand-grip stress in subjects with vasospasm and its relevance in glaucoma. *Invest Ophthalmol Vis Sci*. 2003;44(4):1573-1580.

4. Fuchsjäger-Mayrl G, Wally B, Georgopoulos M, et al. Ocular blood flow and systemic blood pressure in patients with primary open-angle glaucoma and ocular hypertension. *Invest Ophthalmol Vis Sci*. 2004;45(3):834-839.

5. Okuno T, Sugiyama T, Kojima S, Nakajima M, Ikeda T. Diurnal variation in microcirculation of ocular fundus and visual field change in normal-tension glaucoma. *Eye (Lond)*. 2004;18(7):697-702.

6. Evans DW, Harris A, Garrett M, Chung HS, Kagemann L. Glaucoma patients demonstrate faulty autoregulation of ocular blood flow during posture change. *Br J Ophthalmol*. 1999;83(7):809-813.

7. Galambos P, Vafiadis J, Vilchez SE, et al. Compromised autoregulatory control of ocular hemodynamics in glaucoma patients after postural change. *Ophthalmology*. 2006;113(10):1832-1836.

8. Grunwald JE, Riva CE, Stone RA, Keates EU, Petrig BL. Retinal autoregulation in open-angle glaucoma. *Ophthalmology*. 1984;91(12):1690-1694.

9. Feke GT, Pasquale LR. Retinal blood flow response to posture change in glaucoma patients compared with healthy subjects. *Ophthalmology*. 2008;115(2):246-252.

10. Lei Y, Zhang X, Song M, Wu J, Sun X. Aqueous humor outflow physiology in NOS3 knockout mice. *Invest Ophthalmol Vis Sci*. 2015;56(8):4891-4898.

11. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288(5789):373-376.

12. Moncada S, Higgs A. The L-arginine–nitric oxide pathway. *N Engl J Med*. 1993;329(27):2002-2012.

13. Cavet ME, Vittitow JL, Impagnatiello F, Ongini E, Bastia E. Nitric oxide (NO): an emerging target for the treatment of glaucoma. *Invest Ophthalmol Vis Sci*. 2014;55(8):5005-5015.

14. Polak K, Luksch A, Berisha F, Fuchsjaeger-Mayrl G, Dallinger S, Schmetterer L. Altered nitric oxide system in patients with open-angle glaucoma. *Arch Ophthalmol*. 2007;125(4):494-498.

15. Dengel DR, Brown MD, Ferrell RE, Reynolds TH, Supiano MA. A preliminary study on T-786C endothelial nitric oxide synthase gene and renal hemodynamic and blood pressure responses to dietary sodium. *Physiol Res*. 2007;56(4):393-401.

16. Emam WA, Zidan HE, Abdulhalim BE, Dabour SA, Ghali MA, Kamal AT. Endothelial nitric oxide synthase polymorphisms and susceptibility to high-tension primary open-angle glaucoma in an Egyptian cohort. *Mol Vis*. 2014;20:804-811.

17. Liao Q, Wang DH, Sun HJ. Association of genetic polymorphisms of eNOS with glaucoma. *Mol Vis*. 2011;17(17-20):153-158.

18. Nakayama M, Yasue H, Yoshimura M, et al. T-786-->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation*. 1999;99(22):2864-2870.

19. Zago AS, Kokubun E, Fenty-Stewart N, et al. Effect of physical activity and t-786C polymorphism in blood pressure and blood flow in the elderly [in multiple languages]. *Arq Bras Cardiol*. 2010;95(4):510-516.

20. Tunny TJ, Richardson KA, Clark CV. Association study of the 5' flanking regions of endothelial-nitric oxide synthase and endothelin-1 genes in familial primary open-angle glaucoma. *Clin Exp Pharmacol Physiol*. 1998;25(1):26-29.

21. Kang JH, Wiggs JL, Rosner BA, et al. The relation between endothelial nitric oxide synthase gene variants and primary open-angle glaucoma: interactions with gender and postmenopausal hormone use. *Invest Ophthalmol Vis Sci*. 2010;51:971-979.

22. Logan JF, Chakravarthy U, Hughes AE, Patterson CC, Jackson JA, Rankin SJ. Evidence for association of endothelial nitric oxide synthase gene in subjects with glaucoma and a history of migraine. *Invest Ophthalmol Vis Sci*. 2005;46(9):3221-3226.

23. Mojon DS, Hess CW, Goldblum D, et al. Normal-tension glaucoma is associated with sleep apnea syndrome. *Ophthalmologica*. 2002;216(3):180-184.

24. Kaur C, Foulds WS, Ling EA. Hypoxia-ischemia and retinal ganglion cell damage. *Clin Ophthalmol*. 2008;2(4):879-889.

25. Benjamin N, O'Driscoll F, Dougall H, et al. Stomach NO synthesis. *Nature*. 1994;368(6471):502-502.

26. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med*. 2003;9(12):1498-1505.

27. Olson JS, Foley EW, Rogge C, Tsai AL, Doyle MP, Lemon DD. No scavenging and the hypertensive effect of hemoglobin-based blood substitutes. *Free Radic Biol Med*. 2004;36(6):685-697.

28. Duncan C, Dougall H, Johnston P, et al. Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nat Med*. 1995;1(6):546-551.

29. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr*. 2009;90(1):1-10.

30. Lundberg JO, Govoni M. Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic Biol Med*. 2004;37(3):395-400.

31. Li H, Cui H, Kundu TK, Alzawahra W, Zweier JL. Nitric oxide production from nitrite occurs primarily in tissues not in the blood: critical role of xanthine oxidase and aldehyde oxidase. *J Biol Chem*. 2008;283(26):17855-17863.

32. Millar TM, Stevens CR, Benjamin N, Eisenthal R, Harrison R, Blake DR. Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions. *FEBS Lett*. 1998;427(2):225-228.

33. Kozlov AV, Staniek K, Nohl H. Nitrite reductase activity is a novel function of mammalian mitochondria. *FEBS Lett*. 1999;454(1-2):127-130.

34. Hobbs DA, Kaffa N, George TW, Methven L, Lovegrove JA. Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread products in normotensive male subjects. *Br J Nutr*. 2012;108(11):2066-2074.

35. Nicotera P, Bonfoco E, Brüne B. Mechanisms for nitric oxide-induced cell death: involvement of apoptosis. *Adv Neuroimmunol*. 1995;5(4):411-420.

36. Vanhatalo A, Fulford J, Bailey SJ, Blackwell JR, Winyard PG, Jones AM. Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *J Physiol*. 2011;589(pt 22):5517-5528.

37. Weiss J, Frankl SA, Flammer J, et al. No difference in genotype frequencies of polymorphisms of the nitric oxide pathway between Caucasian normal and high tension glaucoma patients. *Mol Vis*. 2012;18(229-31):2174-2181.

38. Zweier JL, Samouilov A, Kuppusamy P. Non-enzymatic nitric oxide synthesis in biological systems. *Biochim Biophys Acta*. 1999;1411(2-3):250-262.

39. Bryan NS, Calvert JW, Elrod JW, Gundewar S, Ji SY, Lefer DJ. Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. *Proc Natl Acad Sci U S A*. 2007;104(48):19144-19149.

40. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med*. 2006;355(26):2792-2793.

41. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov*. 2008;7(2):156-167.

42. Webb A, Bond R, McLean P, Uppal R, Benjamin N, Ahluwalia A. Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. *Proc Natl Acad Sci U S A*. 2004;101(37):13683-13688.

43. Wennmalm A, Benthin G, Edlund A, et al. Nitric oxide synthesis and metabolism in man. *Ann N Y Acad Sci*. 1994;714:158-164.

44. Galassi F, Renieri G, Sodi A, Ucci F, Vannozzi L, Masini E. Nitric oxide proxies and ocular perfusion pressure in primary open angle glaucoma. *Br J Ophthalmol*. 2004;88(6):757-760.

45. Coleman AL, Stone KL, Kodjebacheva G, et al; Study of Osteoporotic Fractures Research Group. Glaucoma risk and the consumption of fruits and vegetables among older women in the Study of Osteoporotic Fractures. *Am J Ophthalmol*. 2008;145(6):1081-1089.

46. Giaconi JA, Yu F, Stone KL, et al; Study of Osteoporotic Fractures Research Group. The association of consumption of fruits/vegetables with decreased risk of glaucoma among older African-American women in the Study of Osteoporotic Fractures. *Am J Ophthalmol*. 2012;154(4):635-644.

47. Barton J, Bain C, Hennekens CH, et al. Characteristics of respondents and non-respondents to a mailed questionnaire. *Am J Public Health*. 1980;70(8):823-825.

48. Grobbee DE, Rimm EB, Giovannucci E, Colditz G, Stampfer M, Willett W. Coffee, caffeine, and cardiovascular disease in men. *N Engl J Med*. 1990;323(15):1026-1032.

49. Kang JH, Loomis SJ, Rosner BA, Wiggs JL, Pasquale LR. Comparison of risk factor profiles for primary open angle glaucoma subtypes defined by pattern of visual field loss: a prospective study. *Invest Ophthalmol Vis Sci*. 2015;56(4):2439-2448.

50. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA Nutrient Database for Standard Reference, Release 28. http://www.ars.usda.gov/nea/bhnrc/ndl. Accessed December 1, 2015.

51. Hu FB, Stampfer MJ, Manson JE, et al. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med*. 1997;337(21):1491-1499.

52. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol*. 1992;135(10):1114-1126.

53. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*. 1985;122(1):51-65.

54. Wu T, Wang Y, Ho SM, Giovannucci E. Plasma levels of nitrate and risk of prostate cancer: a prospective study. *Cancer Epidemiol Biomarkers Prev*. 2013;22(7):1210-1218.

55. Feskanich D, Rimm EB, Giovannucci EL, et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc*. 1993;93(7):790-796.

56. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol*. 1986;124(1):17-27.

57. Cox DR, Oakes D. *The Analysis of Survival Data*. London, England: Chapman and Hall; 1984.

58. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177-188.

59. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics*. 1995;51(2):524-532.

60. Park SC, De Moraes CG, Teng CC, Tello C, Liebmann JM, Ritch R. Initial parafoveal versus peripheral scotomas in glaucoma: risk factors and visual field characteristics. *Ophthalmology*. 2011;118(9):1782-1789.

61. Boo YC, Jo H. Flow-dependent regulation of endothelial nitric oxide synthase: role of protein kinases. *Am J Physiol Cell Physiol*. 2003;285(3):C499-C508.

62. Wan Z, Woodward DF, Stamer WD. Endogenous bioactive lipids and the regulation of conventional outflow facility. *Expert Rev Ophthalmol*. 2008;3(4):457-470.

63. Stamer WD, Lei Y, Boussommier-Calleja A, Overby DR, Ethier CR. eNOS, a pressure-dependent regulator of intraocular pressure. *Invest Ophthalmol Vis Sci*. 2011;52(13):9438-9444.

64. Alm A, Nilsson SF. Uveoscleral outflow: a review. *Exp Eye Res*. 2009;88(4):760-768.

65. Nathanson JA. Nitric oxide and nitrovasodilators in the eye: implications for ocular physiology and glaucoma. *J Glaucoma*. 1993;2(3):206-210.

66. Nathanson JA, McKee M. Alterations of ocular nitric oxide synthase in human glaucoma. *Invest Ophthalmol Vis Sci*. 1995;36(9):1774-1784.

67. Nathanson JA, McKee M. Identification of an extensive system of nitric oxide-producing cells in the ciliary muscle and outflow pathway of the human eye. *Invest Ophthalmol Vis Sci*. 1995;36(9):1765-1773.

68. Schuman JS, Erickson K, Nathanson JA. Nitrovasodilator effects on intraocular pressure and outflow facility in monkeys. *Exp Eye Res*. 1994;58(1):99-105.

69. Buys ES, Ko YC, Alt C, et al. Soluble guanylate cyclase α1-deficient mice: a novel murine model for primary open angle glaucoma. *PLoS One*. 2013;8(3):e60156.

70. Ashpole NE, Overby DR, Ethier CR, Stamer WD. Shear stress-triggered nitric oxide release from Schlemm's canal cells. *Invest Ophthalmol Vis Sci*. 2014;55(12):8067-8076.

71. Lundberg JO, Gladwin MT, Ahluwalia A, et al. Nitrate and nitrite in biology, nutrition and therapeutics. *Nat Chem Biol*. 2009;5(12):865-869.

72. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free Radic Biol Med*. 2010;48(2):342-347.

73. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol (Oxf)*. 2007;191(1):59-66.

74. Webb AJ, Patel N, Loukogeorgakis S, et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension*. 2008;51(3):784-790.

75. Vanhatalo A, Bailey SJ, Blackwell JR, et al. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am J Physiol Regul Integr Comp Physiol*. 2010;299(4):R1121-R1131.

76. Bailey SJ, Fulford J, Vanhatalo A, et al. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol (1985)*. 2010;109(1):135-148.

77. Bailey SJ, Winyard P, Vanhatalo A, et al. Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol (1985)*. 2009;107(4):1144-1155.

78. Presley TD, Morgan AR, Bechtold E, et al. Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide*. 2011;24(1):34-42.

79. Wightman EL, Haskell-Ramsay CF, Thompson KG, et al. Dietary nitrate modulates cerebral blood flow parameters and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *Physiol Behav*. 2015;149:149-158.

80. Park HY, Jung KI, Na KS, Park SH, Park CK. Visual field characteristics in normal-tension glaucoma patients with autonomic dysfunction and abnormal peripheral microcirculation. *Am J Ophthalmol*. 2012;154(3):466-475.e1.

81. Kang JW, Park B, Cho BJ. Comparison of risk factors for initial central scotoma versus initial peripheral scotoma in normal-tension glaucoma. *Korean J Ophthalmol*. 2015;29(2):102-108.

82. Hood DC, Raza AS, de Moraes CG, Liebmann JM, Ritch R. Glaucomatous damage of the macula. *Prog Retin Eye Res*. 2013;32:1-21.

83. Sadun AA. Metabolic optic neuropathies. *Semin Ophthalmol*. 2002;17(1):29-32.

84. Zoumalan CI, Agarwal M, Sadun AA. Optical coherence tomography can measure axonal loss in patients with ethambutol-induced optic neuropathy. *Graefes Arch Clin Exp Ophthalmol*. 2005;243(5):410-416.

85. Loomis SJ, Kang JH, Weinreb RN, et al. Association of *CAV1/CAV2* genomic variants with primary open-angle glaucoma overall and by gender and pattern of visual field loss. *Ophthalmology*. 2014;121(2):508-516.

86. Rothman KJ, Greenland S. *Modern Epidemiology*. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers; 1998.

87. Pennington J. Dietary exposure models for nitrates and nitrites. *Food Control*. 1998;9:385-395.

88. Anjana, Umar S, Iqbal M, Abrol YP. Are nitrate concentrations in leafy vegetables within safe limits? *Curr Sci*. 2007;92:355-360.